



# Glutamate Synthase Microplate Assay Kit

**Catalog # AS0064**

Detection and Quantification of Glutamate Synthase Activity in  
Tissue extracts, Cell lysate Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
VI. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

## I. INTRODUCTION

Glutamine oxoglutarate aminotransferase (abbreviated as GOGAT) is an enzyme involved in synthesis of glutamate from glutamine and  $\alpha$ -ketoglutarate.

The assay is initiated with the enzymatic catalysis of glutamine by GOGAT. The enzyme catalysed reaction products NADH can be measured at a colorimetric readout at 340 nm.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30ml x 4	4 °C
Substrate Dilution	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

### Note:

**Substrate:** add 19 ml Substrate Dilution to dissolve before use, mix.

**Standard:** add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400  $\mu\text{mol/L}$ .

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

#### IV. SAMPLE PREPARATION

##### 1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

##### 2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

## V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 $\mu$ l	--
Distilled water	--	--	200 $\mu$ l
Substrate	190 $\mu$ l	--	--
Sample	10 $\mu$ l	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

**Note:** if the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time.

## VI. CALCULATION

**Unit Definition:** One unit of GOGAT activity is defined as the enzyme decomposes 1 nmol of NADH per minute.

1. According to the protein concentration of sample

$$\text{GOGAT (U/mg)} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times (V_{\text{Sample}} \times C_{\text{Protein}})} \times T$$

$$= 400 \times \frac{(OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{T}{C_{\text{Protein}}}$$

2. According to the weight of sample

$$\text{GOGAT (U/g)} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times (V_{\text{Sample}} \times W / V_{\text{Assay}})} \times T$$

$$= 400 \times \frac{(OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{T}{W}$$

3. According to the quantity of cells or bacteria

$$\text{GOGAT (U/10}^4\text{)} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times (V_{\text{Sample}} \times N / V_{\text{Assay}})} \times T$$

$$= 400 \times \frac{(OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{T}{N}$$

4. According to the volume of sample

$$\text{GOGAT (U/ml)} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} \times T$$

$$= 400 \times \frac{(OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{T}{V_{\text{Sample}}}$$

$C_{\text{Standard}}$ : the standard concentration, 400  $\mu\text{mol/L}$  = 400 nmol/ml;

$V_{\text{Standard}}$ : the volume of standard, 200  $\mu\text{l}$  = 0.2 ml;

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

$W$ : the weight of sample, g;

$N$ : the quantity of cell or bacteria,  $N \times 10^4$ ;

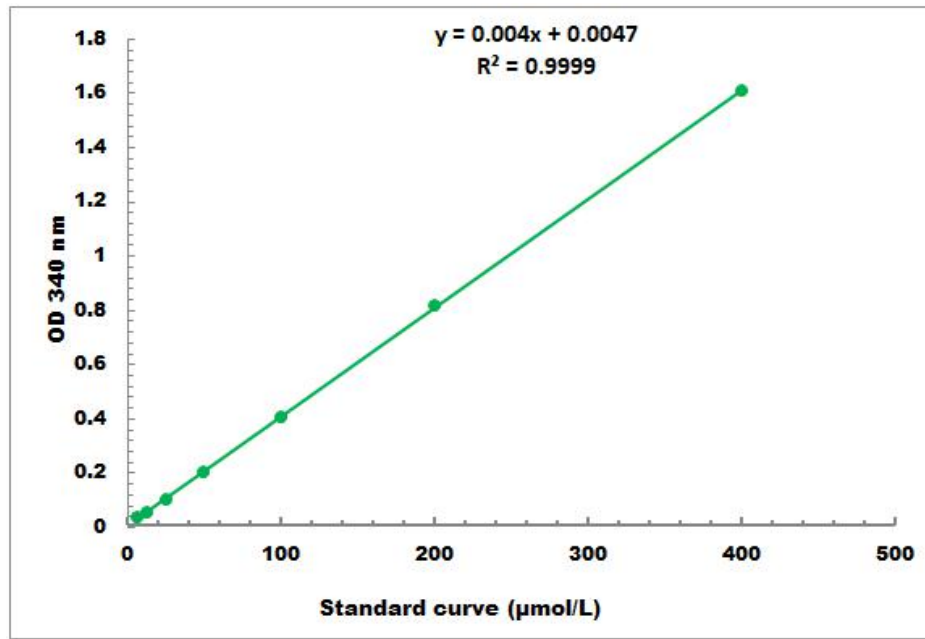
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

$T$ : the reaction time, 2 minutes.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4µmol/L - 400 µmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.sabbiotech.cn](http://www.sabbiotech.cn) or contact us at [techcn@signalwayantibody.com](mailto:techcn@signalwayantibody.com)

## IX. NOTES